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TECHNICAL MANUSCRIPT 340

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Arthur Brown

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DEPARTMENT OF THE ARMY
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Frederick, Maryland

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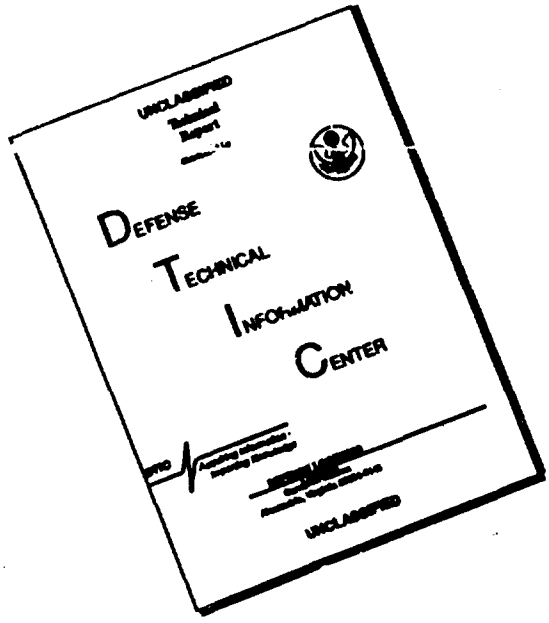


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TECHNICAL MANUSCRIPT 340

# INTERFERENCE AMONG GROUP A ARBOVIRUSES

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BIOLOGICAL SCIENCES LABORATORY

Project 1C014501B71A

January 1967

## ABSTRACT

An interference was observed among different strains of group A arboviruses that did not involve the participation of interferon. To act as an interfering agent, the first virus must be viable and must be inoculated either at a high multiplicity while the superinfecting virus is at a low multiplicity, or the first virus must have several hours' growth advantage in the host cell before superinfection by the second virus. The situation observed for these arboviruses appears to be very similar to that reported for poliovirus. The inhibition by interference might be due to direct competition within the host cell for substrate or replication sites.

## I. INTRODUCTION

Interference of viral growth mediated by interferon has been firmly established. In this type of interference the interfering virus participates only to the extent of inducing the cell to form interferon. The virus itself need not be viable nor grow in the cell and, in fact, non-viral substances are also able to act as interferon inducers. During the past several years a number of reports have appeared that described several other types of interference that are not mediated by interferon. Among these was the interference described by Ledinko in 1963* and Cords and Holland in 1964** among different strains of poliovirus. Replication of interfering virus for a variable length of time in the cell was necessary before the cells became resistant to superinfection with a challenge virus. If the interfering virus was prevented from replicating in the cell, the growth of the challenge virus was not inhibited. It was suggested by Cords and Holland that the interference resulted from replication of the interfering virus in advance of that of the second virus so that many of the substrates or replicating sites within the cell were occupied and not available for the challenge virus.

This report describes an interference between different group A arboviruses that appears to be similar to that observed for poliovirus.

## II. MATERIALS AND METHODS

In most of the experiments reported here the virus used to induce the interference was either the Trinidad strain of Venezuelan equine encephalitis (VEE) virus or a small-plaque variant of this virus designated strain V2. The challenge virus was always eastern equine encephalitis (EEE) virus.

Chick embryo monolayer (CE) cultures were prepared from minced, trypsinized 10-day-old chick embryos. Twenty million to 30 million cells contained in 5 ml of growth medium were placed in 60-mm petri dishes. The growth medium consisted of lactalbumin hydrolyzate, yeast extract, calf serum, and salts. Confluent monolayers were obtained after 24 hours at 37 C in an incubator supplied with a mixture of 5% carbon dioxide in air.

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* Ledinko, N. 1963. An analysis of interference between active polioviruses types 1 and 2 in HeLa cells. Virology 20:29-44.

** Cords, C.E.; Holland, J.J. 1964. Interference between enteroviruses and conditions effecting its reversal. Virology 22:226-234

In those studies involving mixed infection with VEE and EEE viruses, titers in the supernatant growth medium were determined in agar cell suspension cultures to which was added a 1:100 dilution of anti-VEE serum. Plaque formation by VEE virus was inhibited but that of EEE virus was not. This permitted the assay of EEE virus growth in the presence of a large excess of VEE virus. When strain V2 was used, it was not necessary to add antiserum because this virus forms very small plaques and was easily distinguishable from EEE virus in assays of samples from mixed infections.

## III. RESULTS

Interference in the growth of the challenge virus could be demonstrated in two ways. The first was to infect cells with VEE virus several hours before superinfecting the cultures with EEE virus. The second method was to infect the CE cultures simultaneously with both viruses, but to add the interfering virus at a multiplicity of 10 to 100 plaque-forming units per cell, while the challenge virus was used at a 100-fold lower concentration (one plaque-forming unit or less). Regardless of the method used, the degree of interference induced was of similar magnitude.

Figure 1 shows the interference of EEE virus growth that resulted when strain V2 was inoculated onto the cells 3 hours earlier than EEE virus. The EEE virus titer in the doubly infected cultures was 1.6 logs below that observed for the control culture. When the challenge virus was added at times less than 3 hours after the initial infection, there was a corresponding decrease in interference. Maximum interference was observed 3 hours after the cultures were infected with VEE virus.

Superimposed on Figure 1 are two curves for the synthesis of infectious RNA of the challenge virus. In doubly infected cells the amount of infectious RNA was reduced in about the same proportion as that found for the intact virus. These results suggest that the interference observed involved some very early step in synthesis of challenge virus, probably before the virus had the opportunity to synthesize infectious RNA.

Figure 2 shows the effect of infecting CE cultures simultaneously with the two viruses; strain V2 was added at a multiplicity of 100 plaque-forming units per cell with EEE virus at approximately one plaque-forming unit per cell. In the absence of strain V2 virus, EEE grew normally and to high titer; on the other hand, in the presence of the interfering virus, the maximum titer of EEE virus growth was reduced by almost 99%.

These data show that there was a strong inhibition of the growth of the challenge virus. On the other hand, the data on Figure 3 show that the growth of the interfering virus in doubly infected cultures was not affected by the growth of the challenge virus at lower multiplicities and normal growth curves of the former were obtained.

The adsorption and entry of the interfering virus into the host was essential for the development of the interference. If the interfering virus was treated with specific neutralizing antiserum just before infecting CE cells, interference to superinfection with the challenge virus was not observed. Anti-EEE serum incubated with interfering virus (VEE) prior to infection did not prevent the interference. Under these conditions, however, penetration into the host cell of the interfering virus genome also was not sufficient to establish interference. It was possible to inhibit selectively the growth of strain V2 by incubating infected CE cultures at 42 C. The V2 virus genome was not inactivated at this temperature and shifting the cultures back to 37 C permitted the virus to initiate normal growth without lag.

It has been established in previous studies that the V2 virus RNA enters the cell and is maintained in a viable state even for prolonged periods at 42 C. EEE virus, on the other hand, is able to replicate normally at this temperature.

Figure 4 shows the effect of incubating doubly infected cultures at 42 C. The maximum titer of EEE virus was not inhibited even in the presence of strain V2. There was, however, a somewhat reduced rate of EEE virus synthesis during the first 12 hours of infection. These results suggest a necessity for the interfering virus to be able to replicate in the host cell in order to inhibit effectively the growth of the challenge virus.

To help rule out interferon as a factor in the observed interference, actinomycin D was used to inhibit the formation of interferon by virus-infected cells without interfering with arbovirus synthesis. If interferon was involved in the interference observed here, then the challenge virus should be able to grow normally in the presence of actinomycin D.

Table 1 shows that actinomycin D, when added 2 hours prior to infection, had no effect upon the interference of EEE virus in cells that had been previously infected with a high multiplicity of strain V2 virus. In the presence or absence of actinomycin D, the growth of the challenge virus was inhibited to the same extent, about 1.6 logs less than that obtained for the control culture. From these data it seems likely that the interference observed does not result from the formation of interferon by the host cell. Furthermore, when the interfering virus was partially purified by repeated centrifugation to remove any interferon that might be present in the preparation, interference was observed as usual.

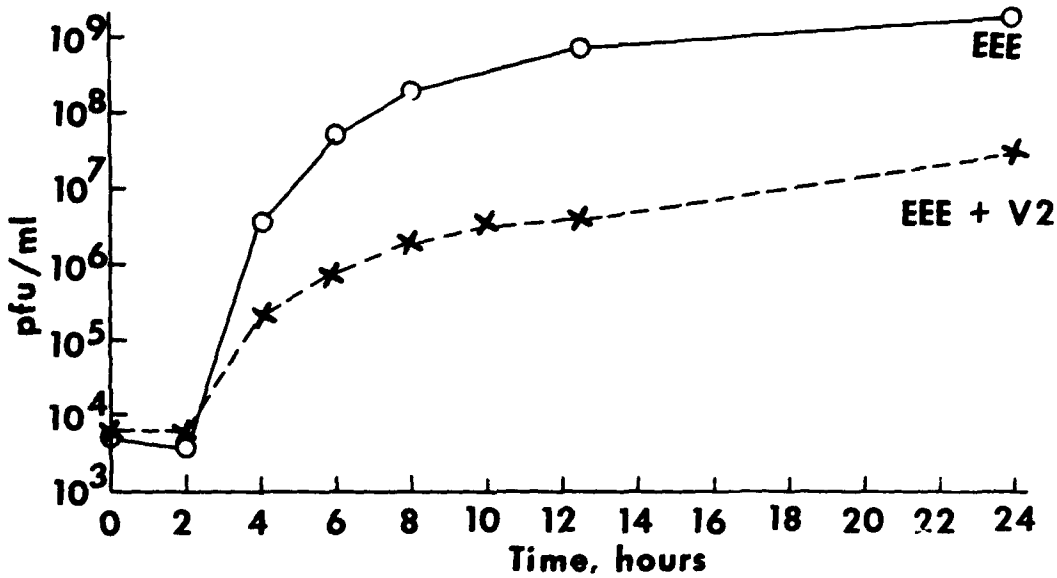


Figure 2. Interference of EEE Virus Growth by V2 at 37 C.

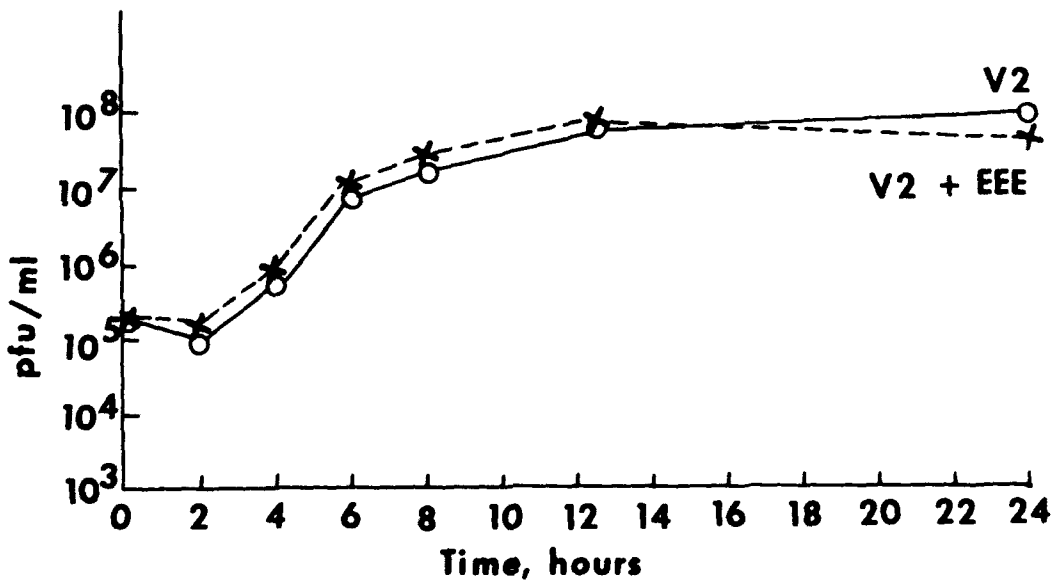


Figure 3. Effect of Mixed Infection with EEE upon Growth of V2 at 37 C.

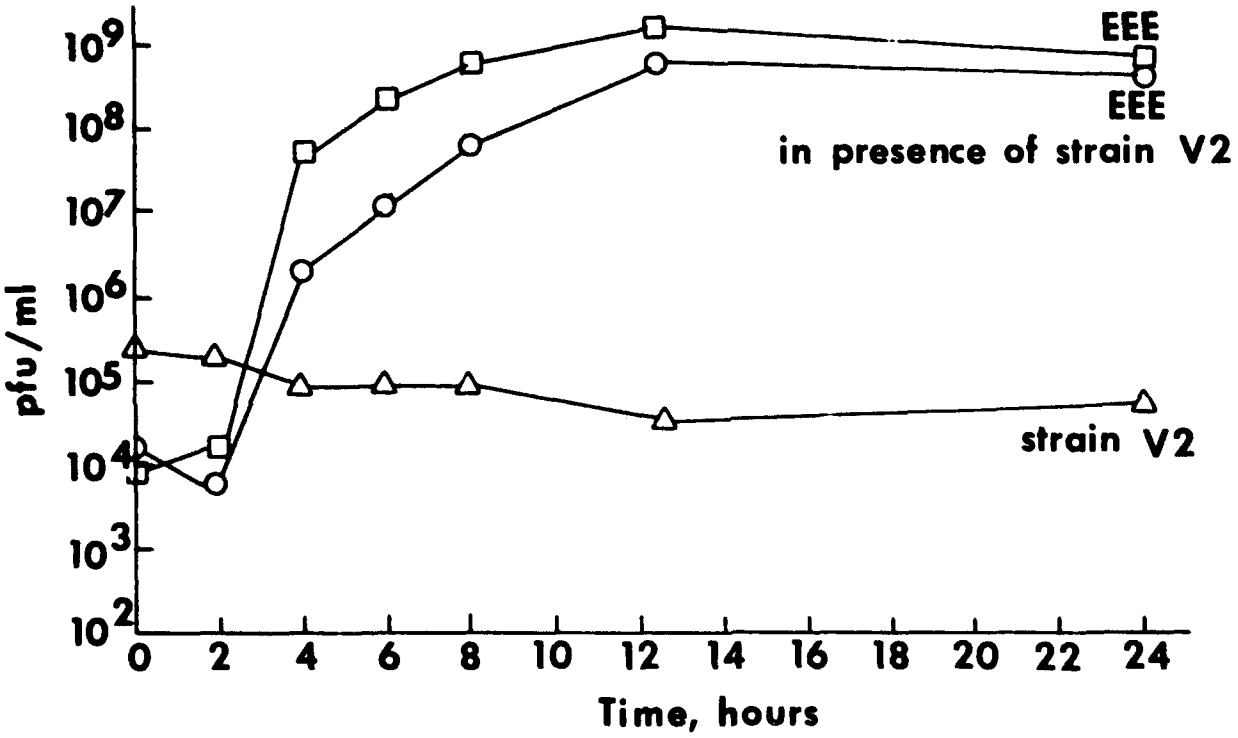


Figure 4. Effect of Incubation at 42 C upon the Abilitv of Strain V2 Virus to Interfere with EEE Virus.

TABLE 1. EFFECT OF ACTINOMYCIN D UPON INTERFERENCE OF EEE VIRUS MULTIPLICATION BY STRAIN V2[a]

| Time, hours | Plaque-Forming Units/ml of EEE Virus | | |
|---|---|---|---|
| | Strain V2 + EEE Virus | Strain V2 + EEE Virus + Actinomycin D[a] | EEE Virus alone |
| 0 | $1.2 \times 10^5$ [b] | $1.2 \times 10^5$ | $1.2 \times 10^5$ |
| 6.5 | $4.1 \times 10^6$ | $6.4 \times 10^6$ | $6.0 \times 10^7$ |
| 22 | $4.7 \times 10^7$ | $4.6 \times 10^7$ | $2.4 \times 10^9$ |

a. Actinomycin D added at 1.0 μg per ml 2 hours before infection.
b. Simultaneous infection of V2 at multiplicity of 100 and EEE at multiplicity of 0.05.

Interference of the growth of EEE virus could be demonstrated when other strains of VEE virus were used. In addition, EEE virus can be used as the interfering virus and can inhibit the growth of a number of tested strains of VEE virus. The interference was reciprocal and could be demonstrated for any combination of viruses used here. However, there seems to be some variation between virus strains in their capacity to serve as interfering viruses. Table 2 shows the results of a typical experiment. The degree of inhibition of EEE virus induced by each VEE virus strain was found to vary. Strain A was the most effective interfering virus, followed by the Trinidad strain of VEE and finally strains V2 and V5. The differences in the degree of inhibition observed may represent differences in the capacity of the different virus genomes to attach to replication sites within the host cell.

TABLE 2. CAPACITY OF DIFFERENT STRAINS OF VEE VIRUS TO INTERFERE WITH THE GROWTH OF EEE VIRUS[a] IN CHICK EMBRYO CELL CULTURE

| | Virus Strain[b] | | | |
|---|---|---|---|---|
| | A | Trinidad | V2 | V5 |
| Degree of interference in $\log_{10}$ units of EEE virus titer | 2.4 | 1.6 | 1.4 | 1.1 |

a. EEE virus used at MOI of 1 pfu per cell or less.
b. VEE virus strains at MOI of 100 pfu per cell.

Regardless of the method used to demonstrate the interference phenomenon, the extent of the interference and the mechanism were probably the same. The simultaneous infection of CE cells with a high multiplicity of interfering virus and low multiplicity of challenge virus gave results that were equivalent to those obtained when interfering virus at high multiplicity was allowed to replicate a few hours before superinfection by challenge virus at the same multiplicity. These results support the thesis that in either case less substrate or fewer replication sites would be available for challenge virus, and that this was most probably the basis of the interference observed. This was further supported by the fact mentioned earlier that all the arbovirus strains used were able to interfere with each other, if the interfering virus was given the advantage of time or higher multiplicity.

In summary, an interference was observed among different strains of group A arboviruses that did not involve the participation of interferon. To act as an interfering agent, the first virus must be viable and must be inoculated either at a high multiplicity while the superinfecting virus is at a low multiplicity, or the first virus must have several hours' growth advantage in the host cell before superinfection by the second virus. The situation observed for these arboviruses appears to be very similar to that reported for poliovirus. The inhibition by interference might be due to direct competition within the host cell for substrate or replication sites.

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14. Key Words

*Arboviruses
*Viral interference
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Eastern equine encephalomyelitis
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Inhibiting
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